UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/759,179	01/20/2004	Chihiro Uematsu	1021.43414X00	7828

20457 7590 07/07/2009

ANTONELLI, TERRY, STOUT & KRAUS, LLP
1300 NORTH SEVENTEENTH STREET
SUITE 1800
ARLINGTON, VA 22209-3873

EXAMINER	
MUMMERT, STEPHANIE KANE	
ART UNIT	PAPER NUMBER
1637	
MAIL DATE	DELIVERY MODE
07/07/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/759,179	UEMATSU ET AL.	
	Examiner	Art Unit	
	STEPHANIE K. MUMMERT	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *16 March 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7 and 10-16* is/are pending in the application.

4a) Of the above claim(s) *10-13* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7 and 14-16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on March 19, 2009 is acknowledged and has been entered. Claim 1 has been amended. Claims 8-9 have been canceled. Claims 1-7, 9 and 14-16 are pending. Claims 10-13 are withdrawn from consideration as being drawn to a non-elected invention.

Claims 1-7 and 14-16 are discussed in this Office action.

All of the amendments and arguments have been thoroughly reviewed and considered but are not found persuasive for the reasons discussed below. Any rejection not reiterated in this action has been withdrawn as being obviated by the amendment of the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This action is made FINAL.

Previous Grounds of Rejection

Priority

Applicant has submitted an English translation of the foreign priority document, Japanese Patent Application No. 114721/2003 filed April 18, 2003. The instant claims will be awarded the filing date of the priority document.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-7, 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Whitcombe et al. (Clinical Chemistry, 1998, 44(5), p. 918-923) in view of Ovyn et al. (6,110,681; August 2000) and Uematsu et al. (2001, Nucleic Acids Research, vol. 29, no. 16, e84, p. 1-6; 'Uematsu 2001' herein). Whitcombe teaches a method of amplification and fluorescence detection using a technique that is 'universal' and can allow for single tube genotyping of more than one target (Abstract, Figure 1).

With regard to claim 1, Whitcombe teaches a method for expressed gene analysis comprising: subjecting a gene to be analyzed to nucleic acid amplification using a forward primer specifically hybridizing to the gene to be analyzed, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, and comprising a second base sequence closer to the 5' end of the primer than the first base sequence (Figure 1, where the forward primer specifically hybridizes to the gene to be analyzed, see black arrow portion of primer comprising the target-specific base sequence; also the primer of Figure 1 comprises a first and second base sequence in addition to the target specific portion), a probe comprising a base sequence identical or complementary to the first base sequence (Figure 1, where the probe is complementary to a base sequence which is non-specific to the target) and

labeled at one end with a fluorophore and at another end with a quencher (Figure 1, where the probe is complementary to a base sequence that is non-specific to the target and where the probe is labeled at one end with a fluorophore and another with a quencher), digesting the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification (bottom of Figure 1, where the probe is digested); and wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes (Figure 2, where more than one probe is used to detect two or more targets in a single tube; see Table 1, where the FAM and TET probes are depicted); and

detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (Figure 2, where the amount of fluorescence emitted by the fluorophore is detected to assay the amount of product of nucleic acid amplification),

wherein the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence (Figure 1, where the forward primer specifically hybridizes to the gene to be analyzed, see black arrow portion of primer comprising the third target-specific base sequence; also the primer of Figure 1 comprises a first and second base sequence in addition to the target specific portion which are nonspecific to the base sequence of the target), and

wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same (Figure 2, where more than one probe is used to detect two or more targets in a single tube; see Table 1, where the FAM and TET probes are depicted).

With regard to claim 15, Whitcombe teaches an embodiment of claim 1, wherein the two or more types of probe respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other (Figure 1, where the probe is complementary to a base sequence that is non-specific to the target and where the probe is labeled at one end with a fluorophore and another with a quencher).

Regarding claim 1, while Whitcombe teaches the simultaneous detection of target genes using two or more types of probes, Whitcombe does not teach that the two or more target genes are derived from different samples. Whitcombe also does not teach the inclusion of reverse transcriptase, RNA polymerase, and ribonuclease H and/or exonuclease and also does not teach that the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence.

With regard to claim 1, Ovyn teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 2) a primer for introduction comprising a second base sequence closer to the 5' end of the primer than the first base sequence (col. 4, lines 48-62, where the primer may include a promoter sequence) 4) reverse transcriptase (col. 2, lines 65-66; see also Figure 1, where the reverse transcriptase is AMV-RT), 5) RNA polymerase (col. 2, lines 65-66, where the RNA polymerase is T7 RNA polymerase, see also Figure 1), and 6) ribonuclease H and/or exonuclease (col. 2, lines 65-66, see Figure 1); and

C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (col. 9, lines 45-55, where detection probes were

hybridized to horseradish peroxidase and the amount of HRP conjugated oligonucleotides was measured to detect target sequence; however as noted at col. 6, lines 30-37, the probe may also be labeled by fluorescent moieties),

wherein the gene to be analyzed is prepared by the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are non-specific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence (see Figure 1 and description recited above).

With regard to claim 2, Ovyn teaches an embodiment of claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction which comprises the first base sequence as described for claim 1 above, in step A) 2) (col. 5, lines 46-64, where primer P1 hybridizes to the RNA to prime and initiate 1st strand synthesis and where after second strand synthesis, the complete cDNA includes the T7 promoter site from the P1 primer).

With regard to claims 3 and 4, Ovyn teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

1) transcription of the gene to analyzed into RNA with the aid of RNA polymerase (col. 5, lines 46-67, see also Figure 1);

2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA (see Figure 1); and

3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase (col. 5, lines 46-67 and Figure 1).

With regard to claim 5, Ovyn teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature (col. 10, lines 15-25, where isothermal amplification of the target RNA was performed at 41°C).

With regard to claim 6, Ovyn teaches an embodiment of claim 5, wherein the single temperature is between 37°C and 55°C (col. 10, lines 15-25, where isothermal amplification of the target RNA was performed at 41°C and wherein 41°C is between 37°C and 55°C).

With regard to claim 7, Ovyn teaches an embodiment of claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence (col. 5, lines 46-64, where primer P1 hybridizes to the RNA to prime and initiate 1st strand synthesis and where after second strand synthesis, the complete cDNA includes the T7 promoter site from the P1 primer; see also Figure 1).

Regarding claim 1, neither Whitcombe nor Ovyn teaches that wherein each of the two or more types of probes comprise several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases.

With regard to claim 1, Uematsu teaches using two or more types of probes to detect two or more target genes simultaneously in a single reaction vessel using two or more types of probes, said two or more target genes are derived from different samples (Figure 2, where two or more probes/primers target two or more target genes which are derived from a mixture of three different samples, A, B and C; see Figure legend which describes "analysis of expressed genes

by one-tube PCR (using MSPs) of the cDNA fragments mixed from different sources"; See Abstract, p. 1, col. 2).

With regard to claim 1, Uematsu also teaches wherein each of the two or more types of probes comprise several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases (Abstract, where the modules were 3 or 4 nt in length; Figure 1, where the primers are comprised of modules consitituted of rearranging the order of the module sequences) and wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same (Figure 1, where the melting temperatures of the primers/probes are identical; Abstract).

With regard to claim 16, Uematsu teaches an embodiment of claim 1, wherein a number of module sequences constituting each probe is in a range of 5 to 8 (Figure 1, where the modular primers/probes included at least six different modular sequences within each primer).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to apply the primer formulation, including a target-specific component and a target non-specific component specific to the probe sequence taught by Whitcombe into the method of NASBA amplification taught generally by Ovyn to arrive at the claimed invention with a reasonable expectation for success. Whitcombe teaches a method called Three-STAR which is "universal in that it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism" and is "particularly useful for the single-tube genotyping of a variety of human DNA polymorphisms" (Abstract). Furthermore, Whitcombe teaches "we have devised a way to make TaqMan generic

in as much that just one fluorogenic probe can be universally applied in any PCR reaction" (p. 921, col. 1-2). Furthermore, the inclusion of a third primer portion, comprising an RNA promoter sequence, would have also been an obvious and necessary substitution to the three-part primer described by Whitcombe for the use of the primers in a NASBA amplification format as taught by Ovyn. Regarding the application of this primer format to additional means of amplification, Ovyn teaches a method known as nucleic acid sequence based amplification (NASBA) (col. 2, lines 46-49). Ovyn describes NASBA as "an amplification system that has significant advantages over PCR amplification systems" because it "requires less user participation and fewer manipulations and steps" and "each cycle of the amplification process generates a plurality of RNA copies from one substrate" (col. 2, lines 49-59). Due to the advantage of generating a plurality of RNA copies, one of ordinary skill would have been motivated minimally to incorporate the features of NASBA taught by Ovyn, into the method of amplification taught by Whitcombe, including incorporating the promoter sequence into the primer for generation of additional RNA copies with a reasonable expectation for success.

Finally, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the module shuffling oligonucleotides of Uematsu into the method of gene expression analysis taught by Ovyn and Whitcombe to arrive at the claimed invention with a reasonable expectation for success. While Uematsu teaches module-shuffling in the form of primers in PCR, the concept of module shuffling is applicable to oligonucleotides, regardless of their intended use as a primer or as a probe. As taught by Uematsu, "competitive PCR amplification of expressed genes from different sources was performed by using 'module-shuffling primers' (MSPs)". Uematu notes "the modules are

arranged in different orders in each primer; therefore, the base sequences of the primers are different, but their melting temperatures are identical" (Abstract). As taught by Uematsu, "we have developed a new method that can analyze plural genes from various sources by utilizing color-selective detection coupled with size separation" (p. 1, col. 2). Furthermore, since Whitcombe teaches multiple probes directed to the detection of multiple sequences in the same sample (Table 1), using probes that are also distinguished by multiple color labeling, Uematsu and Whitcombe share additional features that are applicable to primers or to probes. Therefore, it would have been a simple matter to incorporate the module-shuffling features of Uematsu, in addition to the analysis of a mixed sample comprising multiple individual samples together as taught by Uematsu to the universal probes of Whitcombe to achieve universal detection of multiple target sequences using multiple probes with different sequences with the same thermodynamic properties with a reasonable expectation for success.

Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Whitcombe et al. (Clinical Chemistry, 1998, 44(5), p. 918-923) in view of Ovyn et al. (6,110,681; August 2000) and Uematsu et al. (2001, Nucleic Acids Research, vol. 29, no. 16, e84, p. 1-6; 'Uematsu 2001' herein) as applied to claims 1-7, 15 and 16 above and further in view of Rizzo et al. (Molecular and Cellular Probes, 2002, vol. 16, p. 277-283). Whitcombe teaches a method of amplification and fluorescence detection using a technique that is 'universal' and can allow for single tube genotyping of more than one target (Abstract, Figure 1).

Whitcombe in view of Ovyn and Uematsu teach the limitations of claims 1-7 and 9. Neither Whitcombe, Ovyn or Uematsu teach a probe that is a DNA/RNA hybrid. Rizzo teaches

that the probe for detection is labeled at one end with a quencher and that the probe is digested by ribonuclease H (Figure 1, where the probe is a molecular beacon and is labeled at one end with a quencher and at the other end with a fluorophore).

Regarding claim 14, Rizzo teaches an embodiment of claim 1, wherein at least one of the two or more types of probes is a DNA/RNA hybrid (Figure 1, where the probe is comprised of an RNA:DNA hybrid stem).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of expressed gene analysis taught by Ovyn to incorporate an RNA/DNA hybrid probe of the type disclosed by Rizzo. Rizzo states that "here we describe the preparation of an RNA/DNA chimeric molecular beacon, which contains a single-stranded RNA-DNA chimeric oligonucleotide labeled with a 5' fluorescein as fluorophore and a 3'-DABCYL as quencher (Figure 1). The fluorophore of the probe is held in proximity to the quencher by the stem-loop structure. When the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested." (p. 278, col. 1, 2 paragraph). Furthermore, Rizzo notes that "the chimeric molecular beacon assay for RNAse H is highly sensitive with fluorescent enhancement of up to 40-fold." (p. 282, conclusions). While the chimeric beacon disclosed by Rizzo was not disclosed in the detection of nucleic acids in a format where RNAse H was present, noting the teaching by Rizzo of a molecular beacon with sensitivity to RNAse H activity, it would have been obvious to one of ordinary skill in the art of NASBA amplification, a method in which RNAse H is often included, to incorporate the molecular beacon disclosed by Rizzo into the method of amplification and detection taught by Ovyn with a reasonable expectation for success.

Claims 1-7, 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Whitcombe et al. (Clinical Chemistry, 1998, 44(5), p. 918-923) in view of Leone et al. (1998, Nucleic Acids Research, vol. 26, no. 9, p. 2150-2155) as evidenced by Leone et al. (1997, J. Virol. Methods, 66, 19-27) and in view of Uematsu et al. (2001, Nucleic Acids Research, vol. 29, no. 16, e84, p. 1-6; 'Uematsu 2001' herein). Whitcombe teaches a method of amplification and fluorescence detection using a technique that is 'universal' and can allow for single tube genotyping of more than one target (Abstract, Figure 1).

With regard to claim 1, Whitcombe teaches a method for expressed gene analysis comprising: subjecting a gene to be analyzed to nucleic acid amplification using a forward primer specifically hybridizing to the gene to be analyzed, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, and comprising a second base sequence closer to the 5' end of the primer than the first base sequence (Figure 1, where the forward primer specifically hybridizes to the gene to be analyzed, see black arrow portion of primer comprising the target-specific base sequence; also the primer of Figure 1 comprises a first and second base sequence in addition to the target specific portion), a probe comprising a base sequence identical or complementary to the first base sequence (Figure 1, where the probe is complementary to a base sequence which is non-specific to the target) and labeled at one end with a fluorophore and at another end with a quencher (Figure 1, where the probe is complementary to a base sequence that is non-specific to the target and where the probe is labeled at one end with a fluorophore and another with a quencher), digesting the probe bound

to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification (bottom of Figure 1, where the probe is digested); and

wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes (Figure 2, where more than one probe is used to detect two or more targets in a single tube; see Table 1, where the FAM and TET probes are depicted);

detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (Figure 2, where the amount of fluorescence emitted by the fluorophore is detected to assay the amount of product of nucleic acid amplification),

wherein the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence (Figure 1, where the forward primer specifically hybridizes to the gene to be analyzed, see black arrow portion of primer comprising the third target-specific base sequence; also the primer of Figure 1 comprises a first and second base sequence in addition to the target specific portion which are nonspecific to the base sequence of the target) and

wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same (Figure 2, where more than one probe is used to detect two or more targets in a single tube; see Table 1, where the FAM and TET probes are depicted).

With regard to claim 15, Whitcombe teaches an embodiment of claim 1, wherein the two or more types of probe respectively have fluorophores at one end that emit light at fluorescent wavelengths different from each other (Figure 1, where the probe is complementary to a base

sequence that is non-specific to the target and where the probe is labeled at one end with a fluorophore and another with a quencher).

Regarding claim 1, while Whitcombe teaches the simultaneous detection of target genes using two or more types of probes, Whitcombe does not teach that the two or more target genes are derived from different samples. Whitcombe does not teach that the primer for introduction comprises a second base sequence comprising a promoter sequence of RNA polymerase, which is non-specific to the base sequence of the target gene. Whitcombe also does not teach that the amplification of the gene is accomplished using reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease. Leone discloses the use of molecular beacon probes in the detection of nucleic acids amplified by the NASBA technique (Abstract).

With regard to claim 1, Leone teaches a method for expressed gene analysis, which comprises the steps of: A) subjecting a gene to be analyzed to nucleic acid amplification using 1) a forward primer specifically hybridizing to the gene to be analyzed (p. 2151, col. 1, 'selection of amplification primers and probe' heading, where PD415 or PD416 are antisense primers and PD417 is a sense primer, which were designed to amplify the coat protein open reading frame), 2) a primer for introduction comprising a second base sequence closer to the 5' end of the primer than the first base sequence (p. 2154, Figure 6, legend, where it is noted that the amplicon formed by PD415-PD417 or PD416-PD417 contain a binding site for the T7 RNA polymerase, in addition to complementarity to the target sequence, as described in more detail in Leone et al. 1997, J. Virol. Methods, 66, 19-27, see Table 1, where the sequences of PD415-PD417 are given and also the '*2.2 Selection of amplification primers and detection probe*' heading, where "the antisense ones consisted of a 3' terminal target specific sequence and a 5' terminal T7 promoter

sequence"), 3) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore (p. 2151, col. 1, 'synthesis of the molecular beacons' heading, where a molecular beacon sw75-F1 was designed to bind to nucleotides within the coat protein ORF of PLRV, with DABCYL at the 3' end and fluorescein at the 5' end), 4) reverse transcriptase (p. 2151, 'NASBA' heading, where the reverse transcriptase was included as part of the enzyme mix, which included 6.4 U AMV-reverse transcriptase), 5) RNA polymerase (p. 2151, 'NASBA' heading, where the RNA polymerase is T7 and 32 U are included in the enzyme mix), and 6) ribonuclease H and/or exonuclease (p. 2151, 'NASBA' heading, where 0.08 U RNase H is included in the enzyme mix); and
C) detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification (p. 2151, col. 2 'post-NASBA analysis' and 'Real-time monitoring of NASBA reactions and thermal denaturation profiles' heading, see also Figure 2), wherein the gene to be analyzed is prepared by the introduction of the first base sequence and the second base sequence comprising a promoter sequence of RNA polymerase, which are non-specific to the base sequence of the target gene, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence.

With regard to claim 2, Leone teaches an embodiment of claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using a primer for introduction which comprises the first base sequence as described for claim 1 above, in step A) 2) (see description above for Step A) 2), also p. 2150, col. 1, where the process

of NASBA is described in more detail, where the reaction is based on the concurrent activity of AMV-RT, RNase H and T7 polymerase).

With regard to claims 3 and 4, Leone teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):
1) transcription of the gene to analyzed into RNA with the aid of RNA polymerase;
2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and
3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase (p. 2150, col. 1, where the process of NASBA is described in more detail, where the reaction is based on the concurrent activity of AMV-RT, RNase H and T7 polymerase in repetition and where the activity of these enzymes would include each of the preceeding steps recited, including transcription with an RNA polymerase, reverse transcription, and synthesis of the gene using DNA polymerase).

With regard to claim 5, Leone teaches an embodiment of claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature (p. 2151, 'NASBA' heading, where besides an incubation at 65°C prior to introduction of the enzyme mix, the reaction occurred at 41°C).

With regard to claim 6, Leone teaches an embodiment of claim 5, wherein the single temperature is between 37°C and 55°C (p. 2151, 'NASBA' heading, where besides an incubation at 65°C prior to introduction of the enzyme mix, the reaction occurred at 41°C).

With regard to claim 7, Leone teaches an embodiment of claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter

sequence (p. 2154, Figure 6, legend, where it is noted that the amplicon formed by PD415-PD417 or PD416-PD417 contain a binding site for the T7 RNA polymerase, in addition to complementarity to the target sequence, as described in more detail in Leone et al. 1997, J. Virol. Methods, 66, 19-27, see Table 1, where the sequences of PD415-PD417 are given and also the '*2.2 Selection of amplification primers and detection probe*' heading, where "the antisense ones consisted of a 3' terminal target specific sequence and a 5' terminal T7 promoter sequence").

Regarding claim 1, neither Whitcombe nor Leone teaches that wherein each of the two or more types of probes comprise several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases.

With regard to claim 1, Uematsu teaches using two or more types of probes which detect two or more target genes simultaneously in a single reaction vessel using two or more types of probes, said two or more target genes are derived from different samples (Figure 2, where two or more probes/primers target two or more target genes which are derived from a mixture of three different samples, A, B and C; see Figure legend which describes "analysis of expressed genes by one-tube PCR (using MSPs) of the cDNA fragments mixed from different sources"; See Abstract, p. 1, col. 2).

With regard to claim 1, Uematsu also teaches wherein each of the two or more types of probes comprise several module sequences of 3 or 4 bases, both of the terminal bases of each module sequence are identical to each other and each probe is constituted by rearranging the order of the module sequences having identical terminal bases (Abstract, where the modules were 3 or 4 nt in length; Figure 1, where the primers are comprised of modules consitituted of

rearranging the order of the module sequences) and wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same (Figure 1, where the melting temperatures are identical; Abstract).

With regard to claim 16, Uematsu teaches an embodiment of claim 1, wherein a number of module sequences constituting each probe is in a range of 5 to 8 (Figure 1, where the modular primers included at least six different modular sequences within each primer).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to apply the primer formulation, including a target-specific component and a target non-specific component specific to the probe sequence taught by Whitcombe into the method of NASBA amplification taught generally by Leone to arrive at the claimed invention with a reasonable expectation for success. Whitcombe teaches a method called Three-STAR which is "universal in that it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism" and is "particularly useful for the single-tube genotyping of a variety of human DNA polymorphisms" (Abstract). Furthermore, Whitcombe teaches "we have devised a way to make TaqMan generic in as much that just one fluorogenic probe can be universally applied in any PCR reaction" (p. 921, col. 1-2). Furthermore, the inclusion of a third primer portion, comprising an RNA promoter sequence, would have also been an obvious and necessary substitution to the three-part primer described by Whitcombe for the use of the primers in a NASBA amplification format as taught by Leone. Regarding the application of this primer format to additional means of amplification, Leone teaches a method known as nucleic acid sequence based amplification (NASBA) (p. 2150, Abstract, col. 1). Leone states "with respect to other amplification systems

such as the PCR technique, the ability of NASBA to homogeneously and isothermally amplify RNA analytes (e.g., viral genomic RNA, mRNA or rRNA) extends its application range from viral diagnostics to the indication of biological activities such as gene expression and cell viability" (p. 2150, col. 1). Leone also states that NASBA "results in exponential amplification of RNA and DNA products within 90 min, producing as the major amplification product antisense, single-stranded RNA" (p. 2150, col. 1). Due to the advantage of generating a plurality of RNA copies, one of ordinary skill would have been motivated minimally to incorporate the features of NASBA taught by Leone, into the method of amplification taught by Whitcombe, including incorporating the promoter sequence into the primer for generation of additional RNA copies with a reasonable expectation for success.

Finally, the commonality of detection, as taught by Leone, describing "the coupling of RNA amplification by NASBA with amplicon detection by molecular beacons technology to produce a truly homogeneous RNA assay, called AmpliDet RNA" and report "how molecular beacons improve NASBA, enabling a one-tube assay suitable for high-throughput applications without compromising specificity and sensitivity" (p. 2151, col. 1). Therefore, one of ordinary skill would have been motivated to incorporate the promoter sequence taught by Leone into the primer formation taught by Whitcombe to achieve one-tube, high throughput assay of nucleic acid targets with a reasonable expectation for success.

Finally, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have incorporated the module shuffling oligonucleotides of Uematsu into the method of gene expression analysis taught by Whitcombe and Leone to arrive at the claimed invention with a reasonable expectation for success. While Uematsu teaches

module-shuffling in the form of primers in PCR, the concept of module shuffling is applicable to oligonucleotides, regardless of their intended use as a primer or as a probe. As taught by Uematsu, "competitive PCR amplification of expressed genes from different sources was performed by using 'module-shuffling primers' (MSPs)". Uematu notes "the modules are arranged in different orders in each primer; therefore, the base sequences of the primers are different, but their melting temperatures are identical" (Abstract). As taught by Uematsu, "we have developed a new method that can analyze plural genes from various sources by utilizing color-selective detection coupled with size separation" (p. 1, col. 2). Furthermore, since Whitcombe teaches multiple probes directed to the detection of multiple sequences in the same sample (Table 1), using probes that are also distinguished by multiple color labeling, Uematsu and Whitcombe share additional features that are applicable to primers or to probes. Therefore, it would have been a simple matter to incorporate the module-shuffling features of Uematsu, in addition to the analysis of a mixed sample comprising multiple individual samples together as taught by Uematsu to the universal probes of Whitcombe to achieve universal detection of multiple target sequences using multiple probes with different sequences with the same thermodynamic properties with a reasonable expectation for success.

Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Whitcombe et al. (Clinical Chemistry, 1998, 44(5), p. 918-923) in view of Leone et al. (1998, Nucleic Acids Research, vol. 26, no. 9, p. 2150-2155) as evidenced by Leone et al. (1997, J. Virol. Methods, 66, 19-27) and further in view of Uematsu et al. (2001, Nucleic Acids Research, vol. 29, no. 16, e84, p. 1-6; 'Uematsu 2001' herein) as applied to claims 1-7 and 15-16 above and further in view

of Rizzo et al. (Molecular and Cellular Probes, 2002, vol. 16, p. 277-283). Whitcombe teaches a method of amplification and fluorescence detection using a technique that is 'universal' and can allow for single tube genotyping of more than one target (Abstract, Figure 1).

Whitcombe in view of Leone and Uematsu teach the limitations of claims 1-7 and 15-16. Whitcombe nor Leone or Uematsu teach a probe that is a DNA/RNA hybrid. Rizzo teaches that the probe for detection is labeled at one end with a quencher and that the probe is digested by ribonuclease H (Figure 1, where the probe is a molecular beacon and is labeled at one end with a quencher and at the other end with a fluorophore).

Regarding claim 14, Rizzo teaches an embodiment of claim 1, wherein at least one of the two or more types of probes is a DNA/RNA hybrid (Figure 1, where the probe is comprised of an RNA:DNA hybrid stem).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of expressed gene analysis taught by Leone to incorporate an RNA/DNA hybrid probe of the type disclosed by Rizzo. Rizzo states that "here we describe the preparation of an RNA/DNA chimeric molecular beacon, which contains a single-stranded RNA-DNA chimeric oligonucleotide labeled with a 5' fluorescein as fluorophore and a 3'-DABCYL as quencher (Figure 1). The fluorophore of the probe is held in proximity to the quencher by the stem-loop structure. When the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested." (p. 278, col. 1, 2 paragraph). Furthermore, Rizzo notes that "the chimeric molecular beacon assay for RNAse H is highly sensitive with fluorescent enhancement of up to 40-fold." (p. 282, conclusions). While the chimeric beacon disclosed by Rizzo was not disclosed in the detection of nucleic acids in a

format where RNAse H was present, noting the teaching by Rizzo of a molecular beacon with sensitivity to RNAse H activity, it would have been obvious to one of ordinary skill in the art of NASBA amplification, a method in which RNAse H is often included, to incorporate the molecular beacon disclosed by Rizzo into the method of amplification and detection taught by Leone with a reasonable expectation for success.

Response to Arguments

Applicant's arguments filed March 16, 2009 have been fully considered but they are not persuasive.

Applicant traverses the rejection of claims in view of Whitcombe, Ovyn and Uematsu. Regarding Whitcombe, Applicant argues that "Whitcombe et al. discloses a method whereby a single TaqMan probe can be used for many polymerase chain reactions" and "to identify a means that Amplification Refractory Mutation System (ARMS) could be exploited in a homogeneous, high throughput... manner" and "a way of using a single pair of allele-specific fluorescent probes for any biallelelic polymorphism (p. 11 of remarks). Applicant also notes that "it can either use a single probe for the detection of any one target DNA sequence or a single pair of probes for genotyping any bi-allelic polymorphism" and that "legend of Figure 1 of this article, on page 921, describing that at the start of cycle 4, the temperature switch is activated so that further priming occurs only from the tag" (p. 12 of remarks). Applicant goes on to note that Whitcombe was addressed in the specification and problems associated with the disclosure. Applicant specifically states that "because two pairs of primers are used, i.e., a primer pair of introduction and another pair hybridizing to the Tag sequence of the synthesized DNA, two different thermal

cycles are necessary" (p. 12 of remarks). Applicant goes on to note the features of the instant invention and that the melting temperature of the probes of Whitcombe are different (p. 13 of remarks).

Regarding a combination of Whitcombe with Ovyn and Uematsu, Applicant states "the combine teachings…. Would have neither taught nor would have suggested the presently claimed subject matter" (p. 13-14 of remarks). Applicant summarizes Ovyn and notes, regarding Uematsu "the primers are comprised of modules constituted of rearranged order of the module sequences" and "Fig. 1 of Uematsu 2001 shows primers, which are different from probes. Unlike probes, primers are extended by DNA polymerases". Applicant argues "the primers shown in Fig. 1 of Uematsu '2001 cannot work as a TaqMan probe or a molecular beacon, in which both 5' and 3' ends are required to be labeled with fluorophore, or one of the ends is labeled with a fluorophore and the other is labeled with a quencher" (p. 15-16 of remarks). Applicant also argues that the rejection is a product of hindsight reasoning (p. 16 of remarks).

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

Next, regarding Applicant's arguments against Whitcombe, it is noted that Applicant is arguing features of Whitcombe that, while interesting, do not teach away from the invention as

instantly claimed. First, Applicant argues that Whitcombe requires a temperature switch because there are two primers necessary for the practice of the method. However, it is noted that the method of claim 1, for example, does not require that the method is carried out a single temperature, or that only a single primer is used in the practice of the method. While the method as claimed may only recite a single primer, the method is claimed as comprising that primer, in addition to other elements. Therefore, the inclusion of a "primer of introduction" and a tag specific primer does not in any way teach away from the method as claimed.

Applicant also argues that the method of Whitcombe is used to type an allele in a single sample and is not for detection of more than one target gene, derived from different samples, detected in the method and that the melting temperature is not the same for the two probes of Whitcombe. While Applicant is correct that Whitcombe teaches genotyping of alleles, it is noted in response that Whitcombe uses two or more types of probes to detect two or more target genes simultaneously, both the wild-type and the mutant sequence, in a heterozygous sample. Furthermore, Whitcombe is not the only reference relied upon for teaching of detection of multiple sequences in a sample, simultaneously, using two or more probes with substantially the same melting temperature. As amended above, Uematsu 2001 is applied for a teaching of module shuffled primers to detect multiple sequences from multiple samples, simultaneously and where the primers/probes are designed to have identical melting temperatures. Therefore, Applicant's arguments regarding Whitcombe alone are not persuasive and the rejection of the claims over Whitcombe, Ovyn and Uematsu renders each element of the claims obvious.

Finally, regarding the arguments against Uematsu, Applicant's arguments have been considered, but are not persuasive. As noted in the arguments stated above, and reiterated here,

While Applicant is correct that the primers of Uematsu 2001 would not function as a TaqMan probe, it is noted that the claims are not rejected as being anticipated by either Whitcombe, Ovyn or Uematsu and the references are relied on for their combined teaching. The features of the module-shuffling primers of Uematsu that render them useful and confer the melting temperature feature in a PCR reaction are **equally applicable** to an oligonucleotide in a different format, including as an oligonucleotide probe. A primer and a probe both comprise oligonucleotide sequences, and these sequences are indistinguishable, in general, whether they are intended to be used as a probe, or a primer. Therefore, the module features of Uematsu applied to a primer can obviously be applied to **any** oligonucleotide, including an oligonucleotide used in a probe format (emphasis added).

As noted by Uematsu, the primers are designed specifically so that "the modules are arranged in different orders in each primer; therefore, the base sequences of the primers are different, but their melting temperatures are identical". Therefore, modules can be arranged to achieve identical melting temperatures using the same base composition but different sequences, equally as well in an oligonucleotide that is intended to be used for a primer, as for an oligonucleotide used a probe. In the instant claims, the only difference between a primer and a probe is that the probes are labeled at either end with a fluorophore and a quencher. These features of the probe are taught by Whitcombe's TaqMan probes. Therefore, it would have been obvious to apply the module-shuffling features of the primers of Uematsu to the multiple 'universal' detection probes of Whitcombe which include the features of a TaqMan probe, including the end labels applicant argues are missing from Uemtasu. The teaching of Uematsu

renders the process of module shuffling of oligonucleotides obvious, and can be applied to any oligonucleotide regardless of intended use. The rejection is maintained.

Applicant traverses the rejection of claim 14 over Rizzo and argues that the combined teachings would "have neither disclosed nor would have suggested the presently claimed method, including the two or more types of probes used, with two or more target genes, derived from different samples, being simultaneously detected in a single reaction vessel." (p. 17of remarks).

These arguments are not persuasive for the reasons given above regarding the rejection in view of Whitcombe, Ovyn and Uematsu. The rejection is maintained.

Applicant traverses the rejection of claims over Whitcombe, Leone '98 and Uematsu. The arguments regarding these rejections are largely the same as those presented above in regard to the rejections over Whitcombe, Ovyn and Uematsu.

Applicant traverses the assertion in the previous office action that "Uematsu '2001 teaches wherein the two or more types of 'probes' have substantially the same melting temperature". Applicant argues, again, "this reference discloses primers, not probes, having identical melting temperatures" and "such disclosure in Uematsu '2001, even in combination with the teachings of Whitcombe, et al., and Leone 1998… would have neither taught nor would have suggested the substantially same melting temperature of the two or more probes" (p. 18 of remarks).

As noted in the arguments stated above, and reiterated here, While Applicant is correct that the primers of Uematsu 2001 would not function as a TaqMan probe, it is noted that the

claims are not rejected as being anticipated by either Whitcombe, Leone or Uematsu and the references are relied on for their combined teaching. The features of the module-shuffling primers of Uematsu that render them useful and confer the melting temperature feature in a PCR reaction are **equally applicable** to an oligonucleotide in a different format, including as an oligonucleotide probe. A primer and a probe both comprise oligonucleotide sequences, and these sequences are indistinguishable, in general, whether they are intended to be used as a probe, or a primer. Therefore, the module features of Uematsu applied to a primer can obviously be applied to **any** oligonucleotide, including in a probe format (emphasis added).

As noted by Uematsu, the primers are designed specifically so that "the modules are arranged in different orders in each primer; therefore, the base sequences of the primers are different, but their melting temperatures are identical". Therefore, modules can be arranged to achieve identical melting temperatures using the same base composition but different sequences, equally as well in an oligonucleotide that is intended to be used for a primer, as for an oligonucleotide used a probe. In the instant claims, the only difference between a primer and a probe is that the probes are labeled at either end with a fluorophore and a quencher. These features of the probe(s) are taught by Whitcombe's TaqMan probes. Therefore, it would have been obvious to apply the module-shuffling features of the primers of Uematsu to the multiple 'universal' detection probes of Whitcombe which include the features of a TaqMan probe, including the end labels applicant argues are missing from Uemtasu. The teaching of Uematsu renders the process of module shuffling of oligonucleotides obvious, and can be applied to any oligonucleotide regardless of intended use. The rejection is maintained.

Applicant also reiterates similar arguments against Whitcombe as argued, and addressed, above, in response to the rejection of claims over Whitcombe, Ovyn and Uematsu. These arguments are not persuasive for the same reasons asserted above.

Related Prior Art

1. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Koteler et al. (PNAS, 1993, vol. 90, p. 4241-4245) teaches modular primes assembled from pentamers and hexamers (Abstract). Lanciotti et al. (Journal of Clinical Microbiology, 2001, 39(12), p. 4506-4513) teaches detection of multiple sequences in pooled samples (p. 4508-9 and Table 1). Guiver et al. (FEMS Immunology and Medical Microbiology, 2000, 28, p. 173-179) teaches analysis of multiple genes in a pooled sample (p. 176, col. 2; Table 1).

2. Yu et al. (WO03/038119; published May 2003) disclose a method of amplification of foot and mouth disease virus (FDMV) using NASBA with detection using chemiluminescence. Rossi et al. (US Patent 5,783,391; July 1998) disclose a method of amplification via cylic amplification using reverse transcriptase and T7 RNA polymerase, however the ribonuclease is RNase A. de Barr et al. (2001, Journal of Clinical Microbiology, p. 1895-1902) disclose a method for isothermal amplification to identify multiple subtypes of HIV-1 using NASBA amplification and molecular beacons.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to STEPHANIE K. MUMMERT whose telephone number is (571)272-8503. The examiner can normally be reached on M-F, 9:00-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

SKM
/Stephanie K. Mummert/
Patent Examiner, Art Unit 1637

/GARY BENZION/
Supervisory Patent Examiner, Art Unit
1637